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An Overview of the Acute and Chronic Effects of First and Second Generation Pesticides on an Estuarine Mysid

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16. ABSTRACT

Results are reported for laboratory experiments using the mysid shrimp, Mysidopsis bahia, as a pratical test species for life-cycle studies to determine subtle effects of toxicants. Ratios of the acute 96-hour lethal concentrations to the maximum acceptable toxicant concentration (MATC) ranged from 0.01 to 0.54, indicating that, in some instances, acute tests would be predictive of chronic effects. Criteria used to establish MATC limites were effects on growth, reduced reproduction, chronic toxicity and, with two chemicals, reproduction and chronic toxicity, concurrently. Though the data base is incomplete, the acute tests with M. bahia and 11 pesticides show sensitivities within a factor of 10 to some decapod crustaceans--most of them being penaeid shrimp. Acute studies conducted elsewhere show M. bahia to be as sensitive to, or in many instances, more sensitive than five fresh water and marine species commonly used in toxicity testing.

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AN OVERVIEW OF THE ACUTE AND CHRONIC EFFECTS OF FIRST AND SECOND GENERATION PESTICIDES ON AN ESTUARINE MYSID

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INTRODUCTION

The mysid shrimp, *Mysidopsis bahia*, was introduced as a laboratory organism for life-cycle toxicity testing in 1977 (Nimmo *et al.* 1977). This species has since been used experimentally to determine (1) the toxicity of dredge materials (Shuba *et al.* 1978), (2) the toxicities of several priority pollutants (U.S. EPA 1978), (3) the acute and chronic effects of a metal (Nimmo *et al.* 1978a) and (4) pesticides (Nimmo *et al.* 1977; Nimmo *et al.* 1979; Schimmel *et al.* 1979; and Nimmo *et al.* 1980). In addition, *M. bahia* has been used successfully to determine possible long-term effects due to dredging in estuaries (Nimmo *et al.* in press).

Because some of the research completed with pesticides has not been published elsewhere, this paper will briefly summarize data from eleven studies in which pesticides were used as test toxicants. Additional purposes of this paper are to summarize criteria for determining chronic effects, where possible, to compare the sensitivity of mysids with other species exposed to the same chemicals and, finally, to

discuss acute versus chronic test results with regard to estimated "safe" concentrations of a chemical throughout an entire life cycle.

MATERIALS AND METHODS

Detailed procedures for culturing the mysids and testing protocols have been published previously (Nimmo *et al.* 1978b and 1978c). Mysids, obtained from Santa Rosa Sound near Pensacola, Florida, were cultured by two methods: (1) flowing sea water supplied to 38-liter glass aquaria or a 150-liter circular fiberglass tank, and (2) a static recirculating method in which a biological filter was established in the substratum of a 38-liter aquarium. To provide circulated water, an air-lift tube set in its filter base, was attached to a small glass chamber (vented to the atmosphere) and mounted directly above the aquarium. Currents created by the water flow appeared to aid adult mysids in obtaining food. In all culture devices, larval brine shrimp, *Artemia salina*, were fed to the mysids *ad libitum*.

The mysids were exposed to the eleven pesticides in flowing saltwater; the carrier solvent was triethylene glycol. All tests were conducted in systems delivering intermittent flows from a diluter (Mount and Brungs 1967) or continuous flows with each toxicant added by an infusion pump (Bahner *et al.* 1975). Previous studies have shown these methods to be comparable because approximately equal volumes of water flow through the test aquaria and the desired pesticide concentrations are easily maintained with either system.

The mysids were observed through a life cycle and, in most cases, two life cycles for a total of 28 days. In a temperature range of 22 to 25°C, female mysids usually release multiple broods with the number of juveniles per brood varying from about 3 to 12 per female. Thus, the number of young per female (or reproductive success) is an easily observed effect. Other effects, such as delay in release of young, growth, abnormal behavior, effects on feeding activity and death, were also noted.

The pesticides used in this study are listed in Table 1. DEF® and trifluralin and herbicides, the remaining nine are insecticides. Diazionon®, EPN, leptophos, Methyl parathion® and phorate and organophosphates; Kepone® and Toxaphene® are chlorinated hydrocarbons; Sevin, a carbamate; and Dimilin®, a benzoylphenyl urea.

TABLE 1. Pesticides used in this study and their chemical names.

COMMON NAME OR REGISTERED TRADEMARK	CHEMICAL NAME
DEF	S, S, S-Tributyl phosphorotrithioate
Diazinon	O, O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate
Dimilin	N-[[4-Chlorophenyl) amino]carbonyl] 2, 6-difluorobenzamide
EPN	O-Ethyl O-p-nitrophenyl phenylphosphonothioate
Kepone	Decachloro octahydro-1, 3, 4-metheno-2H-cyclobuta [cd] pentalene-2-one
Leptophos (Phosvel)	O-(4-Bromo-2, 5-dichlorophenyl)O-methylphenylphosphonothioate
Methyl Parathion	O, O-Dimethyl O-p-nitrophenyl phosphorothioate
Phorate (Thimet)	O, O-Diethyl S-(ethylthio)methyl phosphorodithioate
Carbaryl (Sevin)	N-Naphthyl-N-methylcarbamate
Toxaphene (Camphechlor)	Technical chlorinated camphene
Trifluralin (Treflan)	α, α, α-Trifluoro-2, 6-dinitro-N, N-dipropyl-p-toluidine

Test aquaria were sampled to determine concentrations of pesticide. Samples from each test concentration were analyzed at least twice during a 96-hour acute test and at least four times during a life-cycle test of 28 days duration. Results showed a variance of less than 20% for each concentration. One liter samples were extracted with two 100-ml portions of an organic solvent. Petroleum ether was used to extract most of the compounds; methylene chloride was used in Kepone and Sevin analyses; and 1:1 diethyl ether-petroleum ether (V/V) in Dimilin analyses. The extracts were combined and concentrated on a steam table prior to analysis.

Two chromatographic methods were used to separate and quantify the pesticides: high-pressure liquid chromatography (HPLC) with UV-absorption detection and gas-liquid chromatography (GLC) with electron capture and nitrogen phosphorus flame ionization detectors (NPFID). Sevin and Dimilin concentrations were determined by the HPLC method. Phorate concentrations were determined by the GLC method with a NPFID and the others were determined by the GLC method, using electron caputre detectors (Table 2).

STATISTICAL ANALYSES

The LC50s and 95% confidence limits were calculated by linear regression analysis after probit transformation. Abbot's correction factor for adjusting control mortality was used in the statistical program.

The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean between the lowest concentration that had a significant effect and the highest concentration that had no significant effect in the life-cycle test. The most sensitive criterion for effects in the life-cycle test was used to calculate the MATC; i.e., survival, reproduction, or growth. Selection of the most sensitive criterion was based on the results of the statistical analysis of each set of data. The two sample "t" test was used to compare controls and treatment with respect to growth and survival. Analysis of Variance (ANOVA) was used to determine significant differences among the six treatments (five test concentrations and control) with respect to the number of young per female. If the results of the ANOVA showed significant difference among those treatments, multiple comparisons (*post hoc*) tests were made to distinguish between "no effect" and "effect" groups of concentrations by determining nonsignificant group. Student-Newman-Keuls (SNK), Duncan's, Dunnett's, and Bonferrone's tests were used to

TABLE 2. Detection limits and recoveries of pesticides from water.

Compound	Method of Detection	Column and Temperature	Detection Limit (µg/l)	Fortified Recovery (%)	Reference
DEF	GLC/EC[1]	2% SP2100 210°C	0.010	>85	Unpublished
Diazinon	GLC/EC	2% SP2100 175°C	0.020	>90	Goodman 1979
Dimilin	UV-ABSOR[2]	µ NH_2[3]	0.40	>85	Nimmo et al. 1980
EPN	GLC/EC	2% SP2100 210°C	0.20	85	Schimmel et al. 1979
Kepone	GLC/EC	2% SP2100 200°C	0.20	>85	Schimmel and Wilson 1977
Leptophos	GLC/EC	2% SP2100 210°C	0.025	>85	Schimmel et al. 1979
Methyl Parathion	GLC/EC	2% SP2100 210°C	0.10	>85	Unpublished
Phorate	GLC/NPFID	2% SP2100 200°C	0.020	>85	Unpublished
Sevin	UV-ABSOR	µ NH_2	3.0	>95	Unpublished
Toxaphene	GLC/EC	2% SP2100 200°C	0.020	>85	Schimmel et al. 1977
Trifluralin	GLC/EC	5% QF-1 175°C	0.020	>85	Unpublished

[1]GLC/EC, Gas- Liquid Chromatography/Electron-Capture Detector

[2]UV-ABSOR, UV Absorbance Detection

[3]µ NH_2, Micro NH_2 Column

compare treatments and controls with respect to total brood. These statistical tests are similar and have been modified for use in computer programs. Since these studies spanned a period of four years, differing tests were employed as computational equipment and programs became available. Significant differences (α=0.05) were attributed to the effects of the respective pesticide being tested.

RESULTS AND DISCUSSION

Acute Toxicities

All pesticides were acutely toxic to *Mysidopsis bahia*, except trifluralin, which had a 96-hour LC50 greater than 136.0 µg/l (Table 3). Greater test concentrations were not possible due to low solubility in water. Phorate was the most acutely toxic chemical, 96-hour LC50=0.33 µg/l. Therefore, the range of LC50s for these pesticides was three orders of magnitude.

Although the criterion of effect in acute tests was death, observations of the animals exposed to some chemicals indicated stress before death. In tests with Phorate and Methyl parathion, loss of equilibrium, reduced feeding activity and reduced growth (development) occurred. Reduced growth of the juveniles was particularly conspicuous in the higher concentrations of Kepone and DEF. An effect observed only in the Dimilin tests was failure of the mysids to produce a chitinous exoskeleton during the molt.

In acute tests, differences in mortality were noted between juveniles and adults and, in general, juveniles showed the greater sensitivity. Exceptions were that Diazinon and EPN were more acutely toxic to adults than to juveniles; however, these differences were less than a factor of 1/2 or 1/3. The reason for using juveniles instead of adult animals for acute toxicity tests was the minimal effort to standardize the age and sizes of the animals prior to beginning the test. Juveniles 48 hours old or younger, released from adult females, were used to begin each acute and chronic test (Nimmo *et al.*, 1978c).

Two comparisons can be made between the sensitivites of mysids and other species tested with the same pesticide, the first being a comparison between mysids and decapods tested with the pesticides used in this study (Table 4). The 48- or 96-hour LC50s of penaeid shrimp were available with one exception: the stone crab, *Menippe mercenaria*, was exposed to Dimilin from the hatching of the larvae to the first crab

TABLE 3. Summary of toxicity tests using Mysidopsis bahia and eleven pesticides.

Compound	96-Hour LC50 (µg/l) 95% Confidence Limits (juveniles)	MATC Limits (µg/l) (juvenile through adult)	Application Factor[1]
DEF	4.55 (4.21 - 4.92)	<0.34[2]	<0.08
Diazinon	4.82 (4.11 - 5.87)	>1.15 < 3.27	0.54
Dimilin	1.97 (1.73 - 2.24)	<<0.4[2]	<<0.2
EPN	3.01 (2.46 - 3.75)	>0.44 < 4.13	0.45
Kepone	10.29 (8.07 -12.57)	>0.026< 0.34	0.01
Leptophos	3.31 (2.77 - 4.04)	>0.64 < 1.77	0.32
Methyl Parathion	0.77 (0.64 - 0.98)	>0.11 < 0.16	0.17
Phorate	0.33 (0.27 - 0.43)	>0.09 < 0.14	0.33
Sevin	>7.7 --	--	--
Toxaphene	2.67 (1.94 - 4.05)	>0.07 < 0.14	0.04
Trifluralin	>136.4 --	--	--

[1]Derived by dividing the geometric mean of the maximum acceptable toxicant limits by the 96-hour LC50.

[2]A no-effect concentration has not been determined.

TABLE 4. Comparative toxicities of pesticides used in this study to juvenile mysids and some representative marine decapods.

Pesticide	Mysid 96-Hr.LC50 (µg/l)	Species	48-Hr.LC50 (µg/l)	Reference
Phorate	0.33	Penaeus aztecus	0.46	Butler 1964
Methyl Parathion	0.77	Penaeus aztecus	3.2	Butler 1964
Dimilin	1.97	Menippe mercenaria	0.5[2]	Costlow 1979
Toxaphene	2.67	Penaeus aztecus	2.7	Lowe, personal communication[1]
EPN	3.01	Penaeus duorarum	0.29[3]	Schimmel et al. 1979
Leptophos	3.31	Penaeus duorarum	1.88[3]	Schimmel et al. 1979
DEF	4.55	Penaeus aztecus	28.0	Butler 1965
Diazinon	4.82	Penaeus aztecus	28.0	Lowe, personal communication[1]
Sevin	>7.70	Penaeus aztecus	1.5	Butler 1963
Kepone	10.29	Penaeus aztecus	68.0	Butler 1963
Trifluralin	>136.0	Penaeus duorarum	>210.0[3]	Schimmel, personal communication[1]

[1]Personal communication from J. I. Lowe and S. G. Schimmel. Information available from ERL, EPA, Sabine Island, Gulf Breeze, Florida 32561.

[2]Decreased survivorship of larvae.

[3]96-Hr. LC50.

stage (Costlow 1979). The LC50s for the decapods, most of them tested as sub-adults, were within an order of magnitude of those for juvenile mysids. Although these data are not conclusive because of differing procedures, exposure times, and small sample size, they indicate fair agreement in results. One obvious advantage of using mysids rather than juveniles or sub-adults of decapod crustaceans is the mysid's availability throughout the year.

Other investigations showed the mysid's sensitivity compared to other species commonly tested against a variety of chemicals. These studies (U.S. EPA 1978) were conducted to fill informational gaps in water quality criteria documents for both fresh-water and marine species. Results showed that *M. bahia* was equal to, or was the most sensitive species, 52% of the time based on 29 priority pollutants for which comparable data existed. The fresh water species in these tests were the alga, *Selenastrum capricornutum*; a daphnid, *Daphnia magna*; and the bluegill sunfish, *Lepomis macrochirus*. The marine species were: an alga, *Skeletonema costatum;* the mysid, *Mysidopsis bahia;* and the sheepshead minnow, *Cyprinodon variegatus*. The order of decreasing species sensitivity (expressed as LC50s for the crustaceans and fishes and EC50s for the algae) was: *M. bahia* (the most sensitive), followed by *S. costatum, L. macrochirus, D. pulex, S. capricornutum* and *C. variegatus* (the least sensitive).

LIFE CYCLE TESTS

The data listed in Table 3 illustrate the differences in sensitivities of this mysid to chemicals tested for 96 hours compared to tests through an entire life cycle. Few estuarine animals have, historically, been available to biologists for life-cycle testing and the primary purpose of these studies was to generate data for comparisons of acute versus chronic tests with chemicals, and ultimately to predict long-term effects from short-term tests. The Maximum Acceptable Toxicant Concentration (MATC) (Table 3) was calculated for each chemical to provide an estimated concentration considered to be "safe" or incapable of providing an observable or measurable effect throughout an animal's life cycle (Eaton 1973). The Application Factor (AF) (Table 3) is the ratio of the MATC divided by the LC50 and provides a number by which estimates of chronic toxicity can be made for chemicals for which only acute toxicity data exists. It should be pointed out that initially the application factor concept was used

primarily for fresh water fishes because adequate data bases existed for these species.

In our studies with mysids, the end point of effect in the acute tests was death but the criteria to determine the MATC depended upon such responses as reduced growth, decreased number of young per female, or chronic toxicity (Table 5).

In tests using measured concentrations of pesticide, Kepone was the most toxic chemical throughout an entire life cycle and the MATC was based on a measurement of growth (Table 3). Female mysids exposed to 0.072 µg/l Kepone grew about 6% less than control mysids, whereas growth of females exposed to 0.026 µg/l was not significantly different from controls. Also, the average number of young per female was 8.9 in the 0.39 µg/l, compared to 15.3 in the controls. Therefore, the effects of Kepone in reducing both growth and reproduction in our tests were apparently consistent with the findings of Jensen (1958) whose studies with 13 species of malacostracans, including five species of mysids, established that the number of eggs produced per female was a direct function of body length.

The most toxic pesticide, based on nominal concentrations, in a life cycle was Dimilin. This test is distinguished from the test with Kepone because the effects of Dimilin on mysids were observed in concentrations below the limit of detection of our analytical capability. Only 13.5 young per female were produced in an estimated concentration of 0.075 µg/l, whereas 21.4 and 20.9 young per female were produced in controls (Table 6). With Dimilin we observed a significant decrease in reproduction with increasing concentrations of the chemical. Survival was greater than 62% in all concentrations, but the number of juveniles per female was significantly less. Therefore, we have not been able to arrive at a "no effect" concentration of Dimilin.

EPN and Methyl Parathion produced significant effects on both survival and reproduction in the same concentration of pesticide. For example, with continuous exposure to EPN at a concentration of 4.13 µg/l (Table 7), survival was only 32.5%, a significant reduction compared to the 89.7 and greater in all other test concentrations and controls. Also, the number of juveniles per female was only 3.5 compared to the 14.6 and greater in all other tests. Thus, the total numbers of mysids in the test concentration of 4.13 µg/l EPN were reduced by two factors acting in concert: (1) greater mortality of parental stock and (2) decreased production of juveniles by surviving females.

TABLE 5. Criteria used to establish MATC[1] limits for nine pesticides.

TABLE 5. Criteria used to establish MATC[1] limits for nine pesticides.

Compound	Criteria[2]
Kepone	Growth (Development)
DEF	# Young/Female
Dimilin	# Young/Female
Toxaphene	# Young/Female
Leptophos	# Young/Female
Diazinon	Chronic Toxicity
Phorate	Chronic Toxicity
Methyl Parathion	# Young/Female and Chronic Toxicity
EPN	# Young/Female and Chronic Toxicity

[1]MATC, Maximum Acceptable Toxicant Concentration.

[2]Growth, length measurement of mysids from tip of carapace to end of uropod; number young/female, number of young per producing female; chronic toxicity, mortality of parental stock throughout a life cycle.

TABLE 6. Effect of Dimilin® on reproductive success and survival of *Mysidopsis bahia* at 22-26°C and 22-28% salinity.

Nominal Test Concentrations (µg/l)	Percentage Survival Of Adults (Day 28)	Juveniles Per Female (Day 28)
Seawater Control	97.5	21.4
TEG[1] Control	94.5	20.9
0.075	75.5	13.5[2]
0.25	81.5	10.2[2]
0.5	71.0	7.2[2]
0.75	62.0	2.4[2]

[1]Triethylene Glycol Control.

[2]Significantly different from controls at $\alpha = 0.05$ (Dunnett's Test).

TABLE 7. Survival and reproductive success of *Mysidopsis bahia* chronically exposed to EPN in flowing seawater for 28 days at 23 to 24° and 22 to 26% salinity.

Measured Test Concentrations (µg/l)	Percentage Survival Of Adults (Day 28)	Juveniles Per Female (Day 28)
Seawater	89.7	16.1
Triethylene Glycol Control	97.4	14.6
0.057	100.0	18.5
0.076	92.5	14.8
0.44	92.5	15.8
4.13	32.5[1]	3.5[2]

[1]Significantly different from controls (Student's t) $\alpha = 0.05$.

[2]Significantly different from controls at $\alpha = 0.05$ (Dunnett's test).

Application Factors (the ratio of MATC divided by the LC50) ranged from 0.54 for Diazinon to 0.01 for Kepone, indicating that for some of the chemicals such as Diazinon, EPN, leptophos and phorate, a 96-hour acute test is close approximation of the MATC. Interestingly, all but Kepone above are organophosphates with "quick kill" properties. However, making estimates or predictions about the long-term effects from 96-hour LC50 values for some of the other pesticides (DEF, Kepone and Toxaphene), may cause the observer to underestimate the long-term chronic effects on mysids. Research with mysids to date suggests that caution should be taken when estimating application factors without data from laboratory studies. Perhaps with more laboratory experiments, using a variety of chemicals to arrive at variance estimates, a single generic application factor can be computed.

SUMMARY AND CONCLUSIONS

Results of laboratory experiments conducted at the Environmental Research Laboratory for several years indicate that the mysid shrimp, *Mysidopsis bahia*, can be used as a practical test species for life-cycle studies to determine the sublethal effects of toxicants. Ratios of the acute 96-hour lethal concentrations to the maximum acceptable toxicant concentration (MATC) ranged from 0.01 to 0.54 indicating that, in some instances, acute tests would be predictive of chronic effects. Criteria used to establish MATC limits were effects on growth, reduced reproduction, chronic toxicity and, with two chemicals, reproduction and chronic toxicity, concurrently. Though the data base is incomplete, the acute tests with *M. bahia* and eleven pesticides show sensitivities within a factor of 10 to some decapod crustaceans--most of them being penaeid shrimp. Acute studies conducted elsewhere show *M. bahia* to be as sensitive to, or in many instances, more sensitive than five fresh water and marine species commonly used in toxicity testing.

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